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Title of Invention Detection of Very low Quantities of Analyte Bound to a Solid phase

Inventors (please provide full names): Robert J. Obremski, John W. Silzel, Tsong-Ieeh Tsay, Bibijahn Cercek, Charles L. Dodson Shaomin Zhou, Tung Rung Wang, Yagang Li

Earliest Priority Date: 10/24/96

Keywords (include any known synonyms registry numbers, explanation of initialisms):

Lateral flow
Protein Array
Multiple Binding Ratios
or Analyte Binding Array

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L1        330867 S IMMUNOASSAY# OR ASSAY# OR IMMUNOCHEMICAL
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L3           277 S BINDING (2W) PARTNER#
L4             9 S L3 (3A) (TWO OR SECOND)
L5             3 S L4 AND L1
L6             8 S L2 AND L1
L7        188106 S FLUORES? OR CYANINE OR DYE#
L8             1 S (L2 OR L4) AND L7
L9         21736 S SOLID (2W) (PHASE# OR SUPPORT#)
L10            1 S L9 AND (L2 OR L4)
L11         5768 S ANALYTE#
L12        14156 S BINDING (2W) L1
L13           33 S L12 AND L11
L14            5 S L13 AND L7
L15          919 S L11 (4A) (DETECT? OR DETERMIN?)
L16          244 S L15 AND L1
L17           42 S L16 AND L7
L18           14 S L9 AND L17
L19        84634 S FLUORO?
L20            8 S L17 AND L19
L21           20 S L20 OR L18
L22            9 S L5 OR L8 OR L10 OR L14
L23           20 S L21 NOT L22

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L22 ANSWER 1 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1999:180704 BIOSIS
DN PREV199900180704
TI Dynamic interaction between soluble tubulin and C-terminal domains of N-methyl-D-aspartate receptor subunits.
AU van Rossum, Denise (1); Kuhse, Jochen; Betz, Heinrich
CS (1) Department of Neurochemistry, Max Planck Institute for Brain Research, Deutschordenstrasse 46, 60528, Frankfurt am Main Germany
SO Journal of Neurochemistry, (March, 1999) Vol. 72, No. 3, pp. 962-973. ISSN: 0022-3042.
DT Article
LA English
AB The cytoplasmic C-terminal domains (CTs) of the NR1 and NR2 subunits of the NMDA receptor have been implicated in its anchoring to the subsynaptic cytoskeleton. Here, we used affinity chromatography with glutathione S-transferase-NR1-CT and -NR2B-CT fusion proteins to identify novel binding partner(s) of these NMDA receptor subunits. Upon incubation with rat brain cytosolic protein fraction, both NR1-CT and NR2B-CT, but not glutathione S-transferase, specifically bound tubulin. The respective fusion proteins also bound tubulin purified from brain, suggesting a direct interaction between the **two binding partners**. In tubulin polymerization **assays**, NR1-CT and NR2B-CT significantly decreased the rate of microtubule formation without destabilizing preformed microtubules. Moreover, only minor fractions of either fusion protein coprecipitated with the newly formed microtubules. Consistent with these findings, ultrastructural analysis of the newly formed microtubules revealed a limited association only with the CTs of the NR1 and NR2B. These data suggest a direct interaction of the NMDA receptor channel subunit CTs and tubulin dimers or soluble forms of tubulin. The efficient modulation of microtubule dynamics by the NR1 and NR2 cytoplasmic domains suggests a functional interaction of the receptor and the subsynaptic cytoskeletal network that may play a role during morphological adaptations, as observed during synaptogenesis and in adult CNS plasticity.

L22 ANSWER 2 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1998:472964 BIOSIS
DN PREV199800472964
TI Simultaneous multiple **analyte** detection using **fluorescent** peptides and capillary isoelectric focusing.
AU Cruickshank, Kenneth A. (1); Olvera, Joe; Muller, Uwe R.
CS (1) Vysis Inc., 3100 Woodcreek Drive, Downers Grove, IL 60515 USA
SO Journal of Chromatography A, (Aug. 21, 1998) Vol. 817, No. 1-2, pp. 41-47. ISSN: 0021-9673.
DT Article
LA English
AB **Analyte**-specific detection based on the isoelectric point of the detection moiety is a new concept that is under investigation at Vysis. We have developed methods for the synthesis of **fluorescent** synthetic peptides that can be conjugated to bioanalytes such as nucleic acids and antibodies, processed in a hybridization or **binding assay**, and then chemically released prior to detection by

capillary isoelectric focusing (cIEF)-laser-induced **fluorescence** (LIF) detection. A two-step cIEF method in coated capillaries using salt mobilization has been used that produces high peak efficiencies and good assay reproducibility. The concentration by focusing aspect of cIEF, which allows for the entire capillary to be filled with sample, enables detection limits in the pM as opposed to sub-nM level for conventional capillary electrophoresis (CE)-LIF. The simultaneous multiple detection of eleven different focusing entities has been achieved.

L22 ANSWER 3 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1998:263343 BIOSIS
DN PREV199800263343
TI Optical immunoprobe development for multiresidue monitoring in water.
AU Brecht, A. (1); Klotz, A.; Barzen, C.; Gauglitz, G.; Harris, R. D.; Quigley, G. R.; Wilkinson, J. S.; Sztajnbok, P.; Abuknesha, R.; Gascon, J.; Oubina, A.; Barcelo, D.
CS (1) Inst. Physiol. Chem., Univ. Tuebingen, 72076 Tuebingen Germany
SO Analytica Chimica Acta, (April 24, 1998) Vol. 362, No. 1, pp. 69-79. ISSN: 0003-2670.
DT Article
LA English
AB Aquifers used for drinking water production require regular monitoring for organic pollutants. Pollutant levels and pollutant patterns may change rapidly especially in surface water. Monitoring systems capable of unattended and automated operation are desirable e.g. at pumping sites. In this paper we report on a study of the application of immunoanalytical techniques for flexible and automated multiresidue testing. A solid phase **fluorescence** immunoassay with immobilised **analyte** derivate and free, **fluorescence** labelled antibody is used. Two optical transducers were tested: A simple 'slab'-waveguide made of sheet glass and an integrated optical (IO) waveguide. Bulk fluorophore excitation was used to estimate the performance of each transducer. Both transducers allow an antibody surface coverage of less than 1permill of a monolayer of protein to be detected. The direct and covalent immobilisation of **analyte** derivates at the transducer surface for a **binding** inhibition **assay** approach is compared to a competitive assay with immobilisation of **analyte** derivates via an auxiliary antibody conjugate. The use of this auxiliary system allows the testing of different **analytes** at the same transducer surface. Atrazine was selected as a model **analyte** for the first trials. The ELISA type assay gives a test midpoint at 2.2 mug/l and an estimated limit of detection of 0.3 mug/l. The fluoroimmunoprobe with a **binding** inhibition **assay** has a test midpoint for atrazine at about 6 mug/l. In the competitive assay with an auxiliary antibody conjugate signal levels were reduced by a factor of two and competition of free atrazine was poor. Titration with free **analyte** derivate (atrazine caproic acid) confirmed that this may be optimised by changing the competing derivate.

L22 ANSWER 4 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1997:385357 BIOSIS
DN PREV199799684560
TI Competitive-**binding assay** method based on

fluorescence quenching of ligands held in close proximity by a multivalent receptor.
AU Ballerstadt, Ralph; Schultz, J. S. (1)
CS (1) Univ. Pittsburgh, Cent. Biotechnol. Bioeng., 300 Technology Dr., Pittsburgh, PA 15219 USA
SO Analytica Chimica Acta, (1997) Vol. 345, No. 1-3, pp. 203-212. ISSN: 0003-2670.
DT Article
LA English
AB A variant of a **fluorescence** quenching affinity assay is described that is based on intermolecular complexation due to specific interaction between an unmodified multivalent lectin and fluorochrome-labeled dextrans bearing specific sugar ligands (**analyte**-analog). The measuring principle relies on the fact that one portion of the dextran is coupled with an emitter **dye** **fluorescein** isothiocyanate (FITC), and the other one with an acceptor **dye** (isothiocyanate-derivatives of rhodamine). In absence of a specific sugar, the bridging of rhodamine and **fluorescein**-labeled dextrans by the lectin results in the formation of a sandwich-like **fluorescein**-dextran/lectin/rhodamine-dextran complex in which the two forms of dextran are very close together (apprx 5 nm) so that **fluorescence** resonance energy transfer (FRET) occurs between **fluorescein** and rhodamine. Hence the **fluorescence** is quenched. The displacement of dextrans by a specific sugar results in the dissociation of the complex and in an inverse increase in **fluorescence** which is proportional to the sugar concentration. The paper describes experiments proofing the conceptual idea of this **fluorescence** assay on two examples: a glucose and galactose-specific assay system. The glucose-specific assay consisted of Concanavalin A (Con A) and **fluorescein** and rhodamine-labeled dextran (M-r 2000 kDa) grafted with mannose. The galactose-specific assay was composed of Ricinus communis agglutinin (RCAI) and **fluorescein** and rhodamine-labeled dextran (M-r 2000 kDa) grafted with lactose. The reversibility and response time of both assays inside a single dialysis hollow fiber, which was fixed within a flow through cell of a fluorometer, were studied during changes of the sugar concentrations. The response time of the sensor fiber was about 4-5 min. The glucose sensor showed a good measurable **fluorescence** signal over a period of 11 days. The use of this assay for antibody/antigen system is proposed.

L22 ANSWER 5 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1996:519052 BIOSIS
DN PREV199699241408
TI Analyte and label binding **assay** read by flow cytometry.
AU Utgaard, Jon Olav; Frengen, Jomar; Stigbrand, Torgny; Ullen, Anders; Schmid, Ruth; Lindmo, Tore (1)
CS (1) Dep. Physics, Norwegian Univ. Sci. Technol., N-7034 Trondheim Norway
SO Clinical Chemistry, (1996) Vol. 42, No. 10, pp. 1702-1708. ISSN: 0009-9147.
DT Article
LA English
AB A new immunometric two-site sandwich **assay** is introduced, in which a label-scavenging binding partner is added to the sample in addition to the analyte-binding partner. The scavenger binding partner

binds excess label antibody, giving a signal proportional to the amount of excess label antibody in the sample solution. A set of two calibration curves is obtained from the **two binding partners** simultaneously, and a combination of the two signals gives an unambiguous determination of the analyte concentration, even for high analyte concentrations where the hook effect may occur. Two-particle immunofluorometric **assays** developed for placental alkaline phosphatase and human chorionic gonadotropin on the basis of this principle and yielding signals measured by flow cytometry gave rapid results (2 h) and had working ranges in excess of 5 and 6 orders of magnitude for the respective analytes.

L22 ANSWER 6 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1995:125512 BIOSIS
DN PREV199598139812
TI A sequential binding **assay** with a working range extending beyond seven orders of magnitude.
AU Frengen, Jomar; Nustad, Kjell; Schmid, Ruth; Lindmo, Tore (1)
CS (1) Dep. Physics, Univ. Trondheim, NTH, N-7034 Trondheim Norway
SO Journal of Immunological Methods, (1995) Vol. 178, No. 1, pp. 131-140. ISSN: 0022-1759.
DT Article
LA English
AB A new immunometric sequential binding **assay** has been developed in which the sample is first reacted with a **solid phase** binding partner in low concentration, and subsequently with a **second binding partner** at a higher concentration. The amounts of analyte bound to the **two solid phase binding partners** are separately measured, thus establishing a double standard curve. There is a shift between the two standard curves along the concentration axis. Thus an unambiguous determination of analyte concentration is obtained, even in the descending region of the curves where the 'hook' effect causes decreasing signal with increasing analyte concentration. A two-particle immunofluorometric **assay** for AFP based on this principle measured by flow cytometry, resulted in an **assay** with rapid binding (apprx 2 h), a detection limit of 0.1 kIU/l and a working range (0.3 to gt 3 times 10-6 kIU/l) in excess of 7 log-10 orders. **Assay** results compared well with those of an immunoradiometric **assay**.

L22 ANSWER 7 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1991:89101 BIOSIS
DN BA91:47991
TI ICAM-1 CD54 A COUNTER-RECEPTOR FOR MAC-1 CCD11B-CD18.
AU DIAMOND M S; STAUNTON D E; DE FOURGEROLLES A R; STACKER S A; GARCIA-AGUILAR J; HIBBS M L; SPRINGER T A
CS CELL DEV. BIOL., HARVARD MED SCH., BOSTON, MASS. 02115.
SO J CELL BIOL, (1990) 111 (6 PART 2), 3129-3140. CODEN: JCLBA3. ISSN: 0021-9525.
FS BA; OLD
LA English
AB While the leukocyte integrin lymphocyte function-associated antigen (LFA)-1 has been demonstrated to bind intercellular adhesion molecule (ICAM)-1, results with the related Mac-1 molecule have been controversial.

We have used **multiple** cell **binding assays**, purified Mac-1 and ICAM-1, and cell lines transfected with Mac-1 and ICAM-1 cDNAs to examine the interaction of ICAM-1 with Mac-1. Stimulated human umbilical vein endothelial cells (HUVECs), which express a high surface density of ICAM-1, bind to immunoaffinity-purified Mac-1 adsorbed to artifical substrates in a manner that is inhibited by mAbs to Mac-1 and ICAM-1. Transfected murine L cells or monkey COS cells expressing human ICAM-1 bind to purified Mac-1 in a specific and dose-dependent manner; the attachment to Mac-1 is more temperature sensitive, lower in avidity, and blocked by a different series of ICAM-1 mAbs when compared to LFA-1. In a reciprocal assay, COS cells cotransfected with the .alpha. and .beta. chain cDNAs of Mac-1 or LFA-1 attach to immunoaffinity-purified ICAM-1 substrates; this adhesion is blocked by mAbs to ICAM-1 and Mac-1 or LFA-1.

Two color **fluorescence** cell conjugate experiments show that neutrophils stimulated with fMLP bind to HUVEC stimulated with lipopolysaccharide for 24 h in an ICAM-1-, Mac-1-, and LFA-1-dependent fashion. Because cellular and purified Mac-1 interact with cellular and purified ICAM-1, we conclude that ICAM-1 is a counter receptor for Mac-1 and that this receptor pair is responsible, in part, for the adhesion between stimulated neutrophils and stimulated endothelial cells.

L22 ANSWER 8 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1987:251573 BIOSIS
DN BA84:4545
TI FIBER-OPTIC CHEMICAL SENSORS FOR COMPETITIVE BINDING FLUOROIMMUNOASSAY.
AU TROMBERG B J; SEPANIAK M J; VO-DINH T; GRIFFIN G D
CS DEP. CHEM., UNIV. TENNESSEE, KNOXVILLE, TENN. 37996-1600, USA.
SO ANAL CHEM, (1987) 59 (8), 1226-1230.
CODEN: ANCHAM. ISSN: 0003-2700.
FS BA; OLD
LA English
AB This paper describes the development of a fiber-optic chemical sensor based on the principle of competitive-**binding fluorescence immunoassay**. Rabbit immunoglobin G (IgG) is covalently immobilized on the distal sensing tip of a quartz optical fiber. The sensor is exposed to **fluorescein** isothiocyanate (FITC) labeled and unlabeled anti-rabbit IgG. The 488-nm line of an argon-ion laser provides excitation of sensor-bound **analyte**. This results in **fluorescence** emission at the optical fiber's sensing lip. Sensor response is inversely proportional to the amount of unlabeled anti-IgG in the sample. Limits of detection (LOD) vary with incubation time, sample size, and measurement conditions. For 10-.mu.L samples, typical LOD are 25 fmol of unlabeled antibody in a 20-min incubation period. These results indicate that each fiber-optic fluoroimmunosensor can be constructed to perform a single sensitive, rapid, low-volume immunoassay, in in situ or benchtop applications.

L22 ANSWER 9 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1985:413962 BIOSIS
DN BA80:83954
TI SOLVENT PERTURBATION **FLUORESCENCE** IMMUNOASSAY TECHNIQUE.
AU HALFMAN C J; WONG F C L; JAY D W
CS DEPARTMENT OF PATHOLOGY, UNIVERSITY OF HEALTH SCIENCES/THE CHICAGO MEDICAL

SCHOOL, NORTH CHICAGO, ILLINOIS 60064.
SO ANAL CHEM, (1985) 57 (9), 1928-1930.
CODEN: ANCHAM. ISSN: 0003-2700.
FS BA; OLD
LA English
AB The use of **fluorescent dyes** to label **analyte** in ligand **binding assays** affords the possibility of convenient, homogeneous assay. The homogenous response depends upon a significant difference in a **fluorescent** property of bound compared to free labeled **analyte**. Dodecyl sulfate quenches the emission intensity of free **fluorescein** labeled gentamycin without influencing the emission intensity of labeled gentamicin bound to gentamicin antibody. This preferential quenching by detergent is demonstrated to serve as the basis for a homogeneous **fluorescence** immunoassay for gentamicin requiring only simple intensity measurements. The method may be used to measure other **analytes** when it can be demonstrated that the perturbing agent (in this case, detergent) preferentially influences the intensity of free labeled **analyte**. This preferential perturbation may be assured by judicious choice of perturbing agent and labeling fluor so that the interaction between labeled **analyte** and the perturbing agent occurs with the **analyte** moiety and not with the fluor moiety.

L23 ANSWER 1 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1999:317122 BIOSIS
DN PREV199900317122
TI Assessment of an automated **solid phase** competitive **fluoroimmunoassay** for benzoylecgonine in untreated urine.
AU O'Connell, Kevin P.; Valdes, James J.; Azer, Nehad L.; Schwartz, Robert P.; Wright, Jeremy; Eldefrawi, Mohyee E. (1)
CS (1) Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, 655 West Baltimore St., Rm. 4-027, Baltimore, MD, 21201 USA
SO Journal of Immunological Methods, (May 27, 1999) Vol. 225, No. 1-2, pp. 157-169.
ISSN: 0022-1759.
DT Article
LA English
SL English
AB A new **solid phase fluoroimmunoassay** using a fully automated flow **fluorometer** adapted for urinalysis of drug metabolites is described. **Fluorescein**-conjugated benzoylecgonine (FL-BE) and monoclonal antibodies (mAb) against benzoylecgonine (BE) were the reagents used for demonstration. The **solid phase** consisted of anti-BE mAbs immobilized on the surface of polymethyl methacrylate (PMMA) beads. Free BE in solution competed with FL-BE and reduced bead-bound **fluorescence** in a concentration-dependent manner. The binding of FL-BE to the anti-BE mAb reached steady-state within minutes. FL-BE was not bound by uncoated beads nor beads coated with non-specific proteins or IgG. The signal-to-noise ratio was 33, and the sensitivity of the **assay** was 2 ng ml-1 for BE. The effective concentration of BE was 1 to 100 ng ml-1, with an IC50 value of 12 ng ml-1. The mAb showed equal affinities for BE, cocaine, and cocaethylene, but a five order-of-magnitude lower affinity for ecgonine and ecgonine methylester. In a double-blind comparison using clinical urine samples,

the data from this single-step competitive **assay** had excellent agreement with results obtained using a fiber-optic biosensor (FOB), and the EMIT **assay** performed commercially. The **assay** provided kinetic data rapidly and can be used to **detect** small **analytes** for which antibodies and **fluorescein** conjugates are available. The affinity of the mAb for FL-BE, calculated from kinetic analysis of the time course of the on and off reaction, was 2.25 X 10-9 M.

L23 ANSWER 2 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1999:199401 BIOSIS
DN PREV199900199401
TI A liquid chromatographic method for analysis of all-rac-alpha-tocopheryl acetate and retinyl palmitate in medical food using matrix **solid -phase** dispersion in conjunction with a zero reference material as a method development tool.
AU Chase, G. William, Jr. (1); Eitenmiller, Ronald R.; Long, Austin R.
CS (1) Atlanta Center for Nutrient Analysis, U.S. Food and Drug Administration, 60 Eighth St, Atlanta, GA, 30309 USA
SO Journal of AOAC International, (Jan.-Feb., 1999) Vol. 82, No. 1, pp. 107-111.
ISSN: 1060-3271.
DT Article
LA English
AB A liquid chromatographic method is described for analysis of all-rac-alpha-tocopheryl acetate and retinyl palmitate in medical food. The vitamins are extracted from medical food without saponification by matrix **solid-phase** dispersion and chromatographed by normal-phase chromatography with **fluorescence** detection. Retinyl palmitate and all-rac-alpha-tocopheryl acetate are quantitated isocratically with a mobile phase of 0.125% (v/v) and 0.5% (v/v) isopropyl alcohol in hexane, respectively. Results compared favorably with label declarations on retail medical foods. Recoveries **determined** on an **analyte**-fortified zero reference material for a milk-based medical food averaged 98.3% (n = 25) for retinyl palmitate spikes and 95.7% (n = 25) for all-rac-alpha-tocopheryl acetate spikes. Five concentrations were examined for each analyte, and results were linear (r2 = 0.995 for retinyl palmitate and 0.9998 for all-rac-alpha-tocopheryl acetate) over the concentration range examined, with coefficients of variation in the range 0.81-4.22%. The method provides a rapid, specific, and easily controlled **assay** for analysis of retinyl palmitate and all-rac-alpha-tocopheryl acetate in fortified medical foods.

L23 ANSWER 3 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1999:130114 BIOSIS
DN PREV199900130114
TI Selective trace enrichment by immunoaffinity capillary electrochromatography on-line with capillary zone electrophoresis-laser-induced **fluorescence**.
AU Thomas, David H.; Rakestraw, David J.; Schoeniger, Joseph S. (1); Lopez-Avila, Viorica; Van Emon, Jeanette
CS (1) Sandia Natl. Lab., P.O. Box 969 MS 9671, Livermore, CA 94551 USA
SO Electrophoresis, (Jan., 1999) Vol. 20, No. 1, pp. 57-66.
ISSN: 0173-0835.
DT Article

LA English
AB Limited by the lack of a sensitive, universal detector, many capillary-based liquid-phase separation techniques might benefit from techniques that overcome modest concentration sensitivity by preconcentrating large injection volumes. The work presented employs selective **solid-phase** extraction by immunoaffinity capillary electrochromatography (IACEC) to enhance **detection** limits. A model **analyte**, **fluorescein** isothiocyanate (FITC) biotin, is electrokinetically applied to a capillary column packed with an immobilized anti-biotin-IgG support. After selective extraction by the immunoaffinity capillary, the bound analyte is eluted, migrates by capillary zone electrophoresis (CZE), and is detected by laser-induced **fluorescence**. The column is regenerated and reused many times. We evaluate the performance of IACEC for selective trace enrichment of analytes prior to CZE. The calibration curve for FITC-biotin bound versus application time is linear from 10 to 300 seconds. Recovery of FITC-biotin spiked into a diluted urinary metabolites solution was 89.4% versus spiked buffer, with a precision of 1.8% relative standard deviation (RSD).

L23 ANSWER 4 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1999:45152 BIOSIS
DN PREV199900045152
TI Automated method for cleanup and determination of benomyl and thiabendazole in table-ready foods.
AU Levine, Robert A.; Luchtefeld, Ronald G.; Hopper, Marvin L.; Salmon, Garrett D.
CS U.S. Food Drug Administration, Total Diet and Pesticide Res. Cent., PO Box 15905, Lenexa, KS 66285-5905 USA
SO Journal of AOAC International, (Nov.-Dec., 1998) Vol. 81, No. 6, pp. 1217-1223.
ISSN: 1060-3271.
DT Article
LA English
AB An automated **solid-phase** extraction (SPE) cleanup with on-line liquid chromatographic (LC) analysis was developed to determine residues of benomyl (as carbendazim) and thiabendazole in table-ready food items from the U.S. Food and Drug Administration Total Diet Study (TDS). A strong-cation-exchange cleanup of an acetone extract replaces the methylene chloride solvent partitioning steps in the procedure described in the Pesticide Analytical Manual (PAM). LC analysis is accomplished with a C8 analytical column and tandem **fluorescence** and UV **detection**. Recoveries of both **analytes** from 32 representative TDS foods fortified at 0.05 and 0.5 mug/g were determined. Method precision was evaluated with triplicate recovery **assays** on 11 foods fortified at both levels. Accuracy was tested further by assaying 47 foods for incurred residues in parallel with the validated PAM procedure for comparison, and good agreement was found. The automated SPE method reduces solvent consumption, analysis time, and labor.

L23 ANSWER 5 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1998:301726 BIOSIS
DN PREV199800301726
TI Liquid chromatographic method for analysis of all-rac-alpha tocopheryl acetate and retinyl palmitate in milk-based infant formula using matrix **solid-phase** dispersion.
AU Chase, G. William, Jr.; Long, Austin R.
CS U.S. Food Drug Adm., Atlanta Cent. Nutrient Analysis, 60 Eighth St., Atlanta, GA 30309 USA
SO Journal of AOAC International, (May-June, 1998) Vol. 81, No. 3, pp. 582-586.
ISSN: 1060-3271.
DT Article
LA English
AB A liquid chromatographic method is described for analysis of all-rac-alpha-tocopheryl acetate, tocopherols, and retinyl palmitate in milk-based infant formula. The vitamins are extracted from infant formula without saponification by matrix **solid-phase** dispersion and quantitated by normal-phase chromatography with **fluorescence** detection. Retinyl palmitate and vitamin E are quantitated isocratically with mobile phases of 0.125% (v/v) and 0.5% (v/v) isopropyl alcohol in hexane, respectively. Results were similar to the certified and non-certified ranges for all-rac-alpha-tocopheryl acetate, retinyl palmitate, and tocopherols in the infant formula standard reference material (SRM) 1846. Results also compared favorably with the label declaration on a retail infant formula. Recoveries were **determined** on an **analyte**-fortified zero control reference material for milk-based infant formula and averaged 96.8% (n = 30) for retinyl palmitate and 91.5% (n = 25) for all-rac-alpha-tocopheryl acetate. Examination of 5 concentrations for each analyte gave results that were linear (r = 0.999) over the concentration examined, with coefficients of variation ranging from 1.02 to 5.86%. The method provides a rapid, specific, and easily controlled **assay** for analysis of retinyl palmitate and vitamin E in fortified infant formula. Additionally, the method minimizes solvent use by using only 14 mL solvent per extraction.

L23 ANSWER 6 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1998:301721 BIOSIS
DN PREV199800301721
TI Determination of ivermectin in salmon muscle tissue by liquid chromatography with **fluorescence** detection.
AU Rupp, Heidi S. (1); Turnipseed, Sherri B.; Walker, Calvin C.; Roybal, Jose E.; Long, Austin R.
CS (1) U.S. Food Drug Adm., Seattle District Office, 22201 23rd Dr. SE, Bothell, WA 98021-4421 USA
SO Journal of AOAC International, (May-June, 1998) Vol. 81, No. 3, pp. 549-553.
ISSN: 1060-3271.
DT Article
LA English
AB A liquid chromatographic method was developed for determination of ivermectin B1a (IVR) extracted from raw fortified and incurred Atlantic salmon muscle tissues. The method was also used to determine fortified

doramectin (DOR) in Atlantic salmon. Tissue extract was applied to a C8 **solid-phase** extraction (SPE) column, followed by a silica SPE column. Residues in the eluate were treated with trifluoroacetic anhydride and methylimidazole to dehydrate the IVR molecule and form an aromatic **fluorescent** moiety with a trifluoroacetic ester. This product was subsequently treated with ammonium acetate in methanol to cleave the ester and convert the functional group back to a stable alcohol form. The **analytes** were **determined** by **fluorescence** with excitation at 272 nm and emission at 465 nm. A C18 Hypersil column was used for analysis with a mobile phase of acetonitrile-water (90 + 10, v/v) and an oven temperature of 65degreeC. IVR and DOR were determined at 5 fortification levels (1, 5, 10, 20, and 40 ppb). Intra-**assay** absolute recoveries ranged from 75 to 89% for IVR and from 73 to 85% for DOR. Relative standard deviations (RSDs) were <7% in all cases. The limit of detection (3 X baseline noise) was 0.25 ppb extracted from tissue. Incurred tissues had an average concentration of 32 ppb, with an RSD of 3%.

L23 ANSWER 7 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1998:252023 BIOSIS
DN PREV199800252023
TI High sample throughput flow **immunoassay** utilising restricted access columns for the separation of bound and free label.
AU Onnerfjord, Patrik (1); Eremin, Sergei A.; Emneus, Jenny; Marko-Varga, Gyorgy
CS (1) Dep. Analytical Chemistry, Land University, P.O. Box 124, 22100 Lund Sweden
SO Journal of Chromatography A, (March 27, 1998) Vol. 800, No. 2, pp. 219-230.
ISSN: 0021-9673.
DT Article
LA English
AB A flow immunodetection system with high sample throughput capacity is described for the screening of various **analytes**. The **immunochemical detection** principle is based on the chromatographic separation of the formed immunocomplex (AbAg or AbAg*) and the free antigen (Ag) by a restricted access (RA) column, utilising size-exclusion and reversed-phase mechanism. A **fluorescein** labelled analyte (Ag*) was used in the competitive **assay** format with **fluorescence** detection. The speed and simplicity of the **assay** were the greatest advantages. allowing measurement of the analyte to be carried out in less than 1 min. The biocompatibility and capacity of the restricted access material allowed multiple injections of up to 5000, without any breakthrough of the **fluorescent** tracer molecule and thus need for regeneration. The flow **immunoassay** was developed using the well-known atrazine herbicide and some transformation products as model compounds, due to their human toxicity and widespread use. The sample throughput was 80 samples per hour and the detection limits were 1.4 nM (300 pg/ml) for atrazine (Ab I) and 2.3 nM (500 pg/ml) for the sum of triazines (Ab II-III). Different sample matrices, PBS buffer, creek water, and urine were successfully applied in the flow system without the need for any sample handling step. For plasma samples an additional clean-up step using **solid-phase**

extraction had to be included. The resulting detection limits for atrazine in plasma and water samples using this clean-up and trace enrichment procedure were found to be 2 ng/ml and 20 pg/ml, respectively. The analysis could be performed at a sample throughput rate of 400 per 6-h working shift.

L23 ANSWER 8 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1997:434190 BIOSIS
DN PREV199799733393
TI Implementation of affinity **solid-phases** in continuous-flow biochemical detection.
AU Lutz, E. S. M.; Irth, H. (1); Tjaden, U. R.; Van Der Greef, J.
CS (1) Div. Analytical Chemistry, Leiden/Amsterdam Cent. Drug Res., Leiden Univ., PO Box 9502, 2300 RA Leiden Netherlands
SO Journal of Chromatography A, (1997) Vol. 776, No. 2, pp. 169-178. ISSN: 0021-9673.
DT Article
LA English
AB A continuous-flow biochemical detection system is presented which allows the use of **solid-phase** immobilized affinity proteins. The biochemical **detection** is performed by mixing **analyte** with a labelled ligand followed by the addition of **solid-phase** immobilized affinity protein. After a reaction time of 85 s, free and bound label are separated by means of a hollow fibre module. Quantitation of the free label is performed with a conventional flow-through **fluorescence** detector. Total **assay** time amounts to less than 2 min. Biotin was chosen as the model compound using a range of streptavidin-coated **solid-phases** and an antibody-coated **solid-phase** as affinity material, and **fluorescein**-biotin as low-molecular-mass label. The relative standard deviation for twenty repetitive injections was 10.9%. A calibration curve was constructed in the concentration range between 20 and 400 nmol l-1 leading to a correlation coefficient of 0.994. A limit of detection of 8 nmol l-1 was obtained.

L23 ANSWER 9 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1996:386795 BIOSIS
DN PREV199699109151
TI Rapid determination of glufosinate, glyphosate and aminomethylphosphonic acid in environmental water samples using precolumn **fluorogenic** labeling and coupled-column liquid chromatography.
AU Sancho, J. V.; Hernandez, F.; Lopez, F. J.; Hogendoorn, E. A. (1); Dijkman, E.; Van Zoonen, P.
CS (1) Lab. Organic-Anal. Chem., Natl. Inst. Public Health Environ. Prot. (RIVM), P.O. Box 1, 3720 BA Bilthoven Netherlands
SO Journal of Chromatography A, (1996) Vol. 737, No. 1, pp. 75-83. ISSN: 0021-9673.
DT Article
LA English
AB The approach presented in recent work (J.V. Sancho et al., J. Chromatogr. A, 678 (1994) 59) concerning the rapid determination of glufosinate in environmental water samples was successfully applied to the development of efficient procedures including the **assay** of glyphosate and its main metabolite, aminomethylphosphonic acid (AMPA). The methodology

involves two approaches: (i) a multi-residue method allowing the simultaneous **determination** of the three **analytes** in environmental water samples to a level of 1 mu-g/l or (ii) single residue methods focused on the analysis of a single analyte to the sub-mu-g/l level. The procedures involve a precolumn derivatisation step with 9-fluorenylmethylchloroformate (FMOC-Cl) yielding highly **fluorescent** derivatives of the **analytes** which then can be **determined** by coupled-column LC with **fluorescence** detection using a reversed-phase C-18 column (C-1) coupled to a weak ion-exchange column (C-2). The separation power of the first column (C-1) was used to achieve sensitivity, by injecting large volume samples, and automated sample clean-up was achieved by removing the less polar interferences, including the excess of hydrolysed reagent (FMOC-OH). Using these procedures, glufosinate, glyphosate and AMPA were successfully recovered from water samples at 0.50-10 mu-g/l fortification levels, with a sample throughput of at least 40 samples per day.

L23 ANSWER 10 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1996:385153 BIOSIS
DN PREV199699107509
TI Determination of fenbufen and its metabolites in serum by reversed-phase high-performance liquid chromatography using **solid-phase** extraction and on-line post-column ultraviolet irradiation and **fluorescence** detection.
AU Siluveru, Madhusudhan; Stewart, James T. (1)
CS (1) Dep. Med. Chem., Coll. Pharm., Univ. Georgia, Athens, GA 30602-2352 USA
SO Journal of Chromatography B Biomedical Applications, (1996) Vol. 682, No. 1, pp. 89-94.
ISSN: 0378-4347.
DT Article
LA English
AB An improved analytical method for the detection and quantification of fenbufen and its two major metabolites is described. The **assay** consists of reversed-phase high-performance liquid chromatography and post-column irradiation with ultraviolet light and **fluorescence** detection. A highly selective chromatography separation was established on a cyanopropyl column at ambient temperature with a flow-rate of 0.5 ml/min. The analytes of interest were isolated from serum using a Bond-Elut C-18 column with high recovery and selectivity. The **fluorescence** response of all three analytes upon UV irradiation was investigated. The post-column UV irradiation was optimized and the effect of irradiation time on the **fluorescence** response was **determined** for all three **analytes**. The **detection** limits were 10 ng/ml for each analyte using 1 ml of serum. Linear calibration curves from 50 to 375 ng/ml for all three **analytes** show coefficients of **determination** of 0.99. Precision and accuracy of the method were within 3.9-6.5 and 5.1-7.4% for fenbufen, 3.5-6.4 and 4.9-6.3% for metabolite II (expressed as lactone III) and 5.4-7.4 and 2.6-7.4% for metabolite IV, respectively.

L23 ANSWER 11 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1996:119785 BIOSIS
DN PREV199698691920
TI Simultaneous determination of danofloxacin and N- desmethyldanofloxacin in

cattle and chicken edible tissues by liquid chromatography with **fluorescence** detection.
AU Strelevitz, Timothy J.; Linhares, Michael C. (1)
CS (1) Dep. Drug Metabolism, Pfizer Central Res. Div., 118-690, Eastern Point
Rd., Groton, CT 06340 USA
SO Journal of Chromatography B Biomedical Applications, (1996) Vol. 675, No. 2, pp. 243-250.
ISSN: 0378-4347.
DT Article
LA English
AB A rugged, simple, and selective method for the determination of danofloxacin and its primary metabolite, N-desmethyldanofloxacin, in cattle (liver, muscle, kidney, and fat) and chicken (liver and muscle) tissues was developed. The method is selective for danofloxacin and N-desmethyldanofloxacin over other veterinary important **fluoroquinolones**, such as enrofloxacin, ciprofloxacin, norfloxacin, and ofloxacin. Selectivity is achieved through a combination of extraction, chromatography, and **fluorescence detection**. The **analytes** were extracted from homogenized tissues using a methanol-perchloric-phosphoric acid solution. After centrifugation, direct injection of extraction supernate was possible. The limit of quantitation was 20 pg on column. Separation was achieved on an Inertsil C-8 (5 mu-m, 100 ANG) column with dimensions of 250 times 4.6 mm I.D. The mobile phase consisted of 0.05 M phosphate buffer (pH 3.5)-acetonitrile (88:12). A **fluorescence** detector was utilized with an excitation wavelength of 280 nm and an emission wavelength of 440 nm. The **assay** was accurate and reproducible within the range of 10 to 500 ng/g for both danofloxacin and N-desmethyldanofloxacin. Intra-**assay** accuracy was between 98 and 101%, and precision was less than 4%. Inter-**assay** accuracy was between 99 and 102%, while precision was less than 2%. Recoveries for both analytes over the dynamic range were greater than 90% for all the tissues.

L23 ANSWER 12 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1996:102153 BIOSIS
DN PREV199698674288
TI Femtomolar sensitivity using a channel-etched thin film waveguide **fluoroimmunosensor**.
AU Plowman, T. E.; Reichert, W. W. (1); Peters, C. R.; Wang, H. K.; Christensen, D. A.; Herron, J. N.
CS (1) Dep. Biomedical Eng., Duke Univ., Durham, NC 27708-0281 USA
SO Biosensors & Bioelectronics, (1996) Vol. 11, No. 1-2, pp. 149-160.
ISSN: 0956-5663.
DT Article
LA English
AB A dual channel, evanescent **fluoroimmunoassay** format is used to **detect** femtomolar **analyte** concentrations (i.e. less than 1 part per trillion (w/ w)) on an etched channel siliconoxynitride thin film integrated optical waveguide. Two **assays** are used to demonstrate the dose-response behaviour of the sensor: (1) a direct **assay** of a **fluorescently**-labeled protein ligand binding to an immobilized protein receptor, and (2) an indirect sandwich **assay** of a non-**fluorescent** protein ligand binding to an immobilized protein receptor, as detected by the binding of a

fluorescently-labeled secondary receptor protein. A red-emitting **cyanine dye** (Cy-5), which minimized background **fluorescence** and scatter losses of the waveguide, was used in both **assays**. To our knowledge, this is the first report of femtomolar sensitivity in an immunosensing instrument.

L23 ANSWER 13 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1995:484183 BIOSIS
DN PREV199598498483
TI Real-time **fluorescence** detection of RNA amplified by Q-beta replicase.
AU Burg, J. Lawrence (1); Cahill, Patrick B.; Kutter, Michael; Stefano, James E.; Mahan, Donald E.
CS (1) GENE-TRAK Corp., 31 New York Avenue, Framingham, MA 01701 USA
SO Analytical Biochemistry, (1995) Vol. 230, No. 2, pp. 263-272. ISSN: 0003-2697.
DT Article
LA English
AB Amplification of RNA probes by Q-beta replicase can be used to **detect** a wide range of **analytes** with a potential sensitivity of a single molecule. A system has been developed in which Q-beta amplification of midivariant(MDV)-based RNA is measured in real time by **fluorescence**. This was accomplished by including a **fluorescent** intercalating **dye**, propidium iodide, in the reactions and monitoring the **fluorescence** change using a custom **fluorometer**. The time at which **fluorescence** is detectable above background is referred to as the "response time" and is calculated using curvefitting algorithms. A response time is inversely and linearly proportional to the logarithm of the number of template RNA molecules which initiated the reaction. Therefore, this system permits an unknown amount of input RNA probe to be quantified through 11 orders of magnitude when compared to a standard curve. Under the described conditions with MDV RNA, the response time occurs when about 3 times 10-11 RNA molecules are synthesized and occurs within the exponential 'phase of the reaction, before the number of active enzyme molecules are saturated with RNA templates. This system has been used to determine the replication properties of MDV RNA reporter molecules bearing specific probe sequences and to develop hybridization **assays** for the clinical diagnostic field.

L23 ANSWER 14 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1994:230433 BIOSIS
DN PREV199497243433
TI Bioanalysis of digoxin and its metabolites using direct serum injection combined with liquid chromatography and on-line **immunochemical** detection.
AU Oosterkamp, A. J.; Irth, H. (1); Beth, M.; Unger, K. K.; Tjaden, U. R.; Van De Greef, J.
CS (1) Leiden/Amsterdam Center Drug Research, Div. Analytical Chem., Univ. Leiden, P.O. Box 9502, 2300 RA Leiden Netherlands
SO Journal of Chromatography B Biomedical Applications, (1994) Vol. 653, No. 1, pp. 55-61.
DT Article

LA English
AB An automated dual-column liquid chromatographic **assay** for digoxin is described. Serum samples are directly injected onto a restricted-access **solid-phase** extraction **support**. After liquid chromatographic (LC) separation on a C-18 analytical column, antigenic **analytes** are **detected** by means of post-column **immunochemical** detection (ICD) using **fluorescein**-labelled antibodies against digoxigenin. The detection limit of this **assay** is 160 pg/ml (preconcentration of 1.0 ml serum). With the present method digoxin and three of its cross-reactive metabolites were determined in serum taken from patients which were orally administered a 1-mg dose of digoxin. The results obtained with LC-ICD were compared with data provided by a batch **immunoassay**.

L23 ANSWER 15 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1994:67331 BIOSIS
DN PREV199497080331
TI A high performance liquid chromatographic method for the determination of albuterol enantiomers in human serum using **solid phase** extraction and a sumichiral-OA chiral stationary phase.
AU Adams, Amanda G. (1); Stewart, James T.
CS (1) Dep. Chem., North Georgia, Coll., Dahlonega, GA USA
SO Journal of Liquid Chromatography, (1993) Vol. 16, No. 17, pp. 3863-3875. ISSN: 0148-3919.
DT Article
LA English
AB A chiral high performance liquid chromatographic method was developed for the simultaneous **assay** of S(+) and R(-) albuterol in human serum. The **assay** utilizes **solid-phase** extraction on a silica column as a sample clean-up step. The chiral separation was accomplished under isocratic conditions using a Sumichiral OA 4700 column and a mobile phase consisting of 350:410:40:2 v/v/v/v hexane/methylene chloride/absolute methanol/trifluoroacetic acid at a flow rate of 1.0 mL/min. The enantiomers were measured using **fluorescence** detection set at 228 nm excitation and an emission filter of gt 280 nm. Racemic atenolol was used as internal standard. Drug to internal standard peak height ratios were linear over a 2-20 ng/mL range for each enantiomer. The limit of **detection** of each **analyte** was 2.0 ng/mL (S/N = 3). The lowest quantifiable level of each enantiomer was 3 ng/mL.

L23 ANSWER 16 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1993:278400 BIOSIS
DN PREV199396008625
TI Liposome-based flow-injection **immunoassay** for determining theophylline in serum.
AU Locascio-Brown, Laurie (1); Plant, Anne L. (1); Chesler, Ruth; Kroll, Martin; Ruddel, Mark; Durst, Richard A.
CS (1) Natl. Inst. Standards and Technol., Gaithersburg, MD 20899 USA
SO Clinical Chemistry, (1993) Vol. 39, No. 3, pp. 386-391. ISSN: 0009-9147.
DT Article
LA English
AB We developed a method for quantitatively determining theophylline in

serum, using a heterogeneous **immunoassay** called flow-injected immunoanalysis. The reaction involves competition between serum theophylline and theophylline-labeled liposomes. Separation occurs on a **solid-phase** reactor column containing immobilized antibody to theophylline incorporated in a flow-injection system. Subsequent lysis of the bound liposomes provides sensitive **detection** of the **analyte**. Effective regeneration of the immobilized antibody activity allows the reactor to be reused for hundreds of sequential samples. Comparison of the results of the flow-injection **immunoassay** method with results obtained with a commercially available **fluorescence** polarization method showed an excellent correlation.

L23 ANSWER 17 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1993:122026 BIOSIS
DN PREV199395066126
TI A high performance liquid chromatographic method for the determination of albuterol enantiomers in human serum using **solid phase** extraction and chemical derivatization.
AU He, Langchong; Stewart, James T. (1)
CS (1) Dep. Med. Chem., Coll. Pharm., Univ. Ga., Athens, Ga. 30602 USA
SO Biomedical Chromatography, (1992) Vol. 6, No. 6, pp. 291-294.
ISSN: 0269-3879.
DT Article
LA English
AB A high performance liquid chromatographic method was developed for the simultaneous **assay** of R(-)- and S(+)-albuterol in human serum. The **assay** involves **solid phase** extraction as a sample clean-up step and derivatization of racemic albuterol to its diastereomeric thioureas with 2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranosyl isothiocyanate. Chromatographic separation was accomplished under isocratic conditions using an octadecylsilane column and a mobile phase consisting of 29:71 acetonitrile:distilled water containing 0.1% triethylamine, pH 4.0 (adjusted with concentrated phosphoric acid) at a flow rate of 0.8 mL/min. The diastereomers were detected using a **fluorescence** detector set at 223 nm excitation and no emission filter. Racemic bamethane was used as internal standard. Drug to internal standard peak-height ratios were linear over a 2-20 ng/mL range for each enantiomer. The limit of **detection** of each **analyte** was 1.0 ng/mL (S/N = 3).

L23 ANSWER 18 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1992:372404 BIOSIS
DN BA94:54454
TI **ASSAY** OF PROTEIN DRUG SUBSTANCES PRESENT IN SOLUTION MIXTURES BY **FLUORESCAMINE** DERIVATIZATION AND CAPILLARY ELECTROPHORESIS.
AU GUZMAN N A; MOSCHERA J; BAILEY C A; IQBAL K; MALICK A W
CS PHARMACEUTICAL RESEARCH DEVELOPMENT, HOFFMANN-LA ROCHE, NUTLEY, N.J. 07110, USA.
SO J CHROMATOGR, (1992) 598 (1), 123-131.
CODEN: JOCRAM. ISSN: 0021-9673.
FS BA; OLD
LA English
AB A method is described to enhance the resolution and detection sensitivity of proteins, peptides, and amino acids in capillary electrophoretic

analysis of solution mixtures. The method consists of derivatizing the analytes with **fluorescamine**, which is normally used as a **fluorogenic** reagent for compounds containing a reactive primary amine functional group, and then using the derivative as an ultraviolet chromophore to enhance detection sensitivity (measured at 280 nm) in capillary electrophoresis. The results demonstrated a significant improvement in the separation and **detection** sensitivity of the derivatized **analytes** as compared to their underivatized counterparts. The use of chromophores, such as **fluorescamine**, in capillary electrophoresis facilitates the analysis of components of solution mixtures, such as pharmaceutical formulations, that could not be resolved and or detected by conventional capillary electrophoresis procedures.

L23 ANSWER 19 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1991:159414 BIOSIS
DN BA91:85214
TI 3-P HYDROXYPHENYLPROPIONIC ACID A SENSITIVE **FLUOROGENIC** SUBSTRATE FOR AUTOMATED **FLUOROMETRIC** ENZYME **IMMUNOASSAYS**
.
AU TUUMINEN T; PALOMAKI P; RAKKOLAINEN A; WELIN M-G; WEBER T; KAPYAHO K
CS LABSYSTEMS OY, PULTTITIE 8, 00880 HELSINKI, FINLAND.
SO J IMMUNOASSAY, (1991) 12 (1), 29-46.
CODEN: JOUIDK. ISSN: 0197-1522.
FS BA; OLD
LA English
AB The application of 3-p-hydroxyphenylpropionic acid (HPPA), a **fluorogenic** substrate of horseradish peroxidase (HRP) to an automated microplate **fluorometric** enzyme **immunoassay** is described. **Fluorescence** intensity of the end product was highly dependent on the pH of the buffer and on the concentrations of the substrate mixture ingredients. The determination of human thyrotropin (TSH) and recombinant hepatitis B surface antigen (rHBsAg) were performed using a **fluorometric** enzyme **immunoassay** (FEIA) with HPPA as the substrate, and a colorimetric one with tetramethylbenzidine (TMB) as the chromogenic substrate. The sensitivity of both types of **assays** proved comparable. The distinct advantage of a **fluorometric assay** is the possibility to perform a quantitative **detection** of **analyte** over a very wide dynamic range. Clinical evaluation of both **assays** showed good correlation between the FEIA and conventional methods.

L23 ANSWER 20 OF 20 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1985:277603 BIOSIS
DN BA79:57599
TI DETERMINATION OF HORMONES BY TIME-RESOLVED **FLUOROIMMUNOASSAY**.
AU LOVGREN T; HEMMILA I; PETTERSSON K; ESKOLA J U; BERTOFT E
CS WALLAC BIOCHEMICAL LABORATORY, P. O. BOX, SF-20101 TURKU 10, FINLAND.
SO TALANTA, (1984) 31 (10B), 909-916.
CODEN: TLNTA2. ISSN: 0039-9140.
FS BA; OLD
LA English
AB **Immunoassays** based on europium labels and time-resolved **fluorescence** as the detection method were developed. The specific activity of the label is several orders of magnitude higher than that of radioactive labels. Consequently, the technique provides great potential, especially in the **determination** of **analytes** which

require high sensitivity. Both competitive and immunometric **assays** which use labeled antibodies have been worked out. In competitive **assays** the antigen is immobilized on a **solid phase** with a protein carrier. The antigen in the standard or sample then competes with the labeled antibody in solution. Separation is done simply by washing the wells in the microtiter strip where the **assays** are performed. Model systems are described for the measurement of testosterone and cortisol. Immunometric **assays** of human TSH (hTSH) and luteotropin (LH) were performed with monoclonal antibodies, by either a one-step (hTSH) or two-step (LH) incubation procedure. These **assays**, which exploit the specific activity of the label, give a very high sensitivity and good reproducibility. The standard curves are linear and the dynamic range is at least 1000-fold. Because of the properties of the europium label and the simple **assay** design, the **immunoassays** based on time-resolved **fluorescence** are expected to gain wide application both in research and in routine determinations.